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STUDIES OF THE LIFE HISTORY OF USTILAGO AVENAE (PERS.) JENSEN AND OF USTILAGO LEVIS (KELL. & SWING.) MAGN.

GEORGE RAYMOND GAGE

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STUDIES OF THE LIFE HISTORY OF USTILAGO AVENAE (PERS.) JENSEN AND OF USTILAGO LEVIS (KELL. & SWING.) MAGN.[1]

GEORGE RAYMOND GAGE

Although seed treatment for the control of the two smuts of oats caused by *Ustilago avenae* (Pers.) Jensen and *U. levis* (Kell. & Swing.) Magn. has been developed to such a point that it is generally regarded as fairly successful, it seems that the ultimate and most economical control of these smuts should lie in the selection or the breeding of resistant varieties. That this is the opinion of most cereal pathologists is evidenced by the marked activity along this line in recent years, as shown by the work of Zavitz (1909, 1914, 1915), Heald (1919), Stapledon (1920), Reed and others (1920, 1924, and 1925), Zade (1922), Arland (1924), Roesch (1926), and many others. Successful procedure toward such an end necessitates not only a thorough knowledge of the physiology and anatomy of the suscept, but also a rather detailed and exact understanding of the life histories of the pathogenes. If one is to test varieties for susceptibility or resistance, he must, above all, understand and be able to manipulate those factors which condition infection.

Because of the many conflicting ideas of various investigators, and especially because of the recent pronouncements on blossom infection made by Zade and his students, it has seemed advisable to reinvestigate the life histories of the pathogenes causing the smut diseases of oats. It is very evident that the life histories of these pathogenes—*Ustilago avenae*, the rough-spored species causing the so-called "loose smut," and *U. levis*, the smooth-spored species causing the so-called "covered smut"—are not adequately understood.

It is not the intention of the writer to bring together into an historical account the development of the present-day ideas on the life histories of these two pathogenes. It will be noticed at once by one who delves into the history of the subject that the writings of the earlier investigators are very confusing and indefinite. All of the loose smuts of cereals were at first considered to be caused by one and the same pathogene, commonly called *Ustilago carbo* or *U. segetum*. It was not until 1888 that

[1]Also presented to the Faculty of the Graduate School of Cornell University, August, 1926, as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

AUTHOR'S ACKNOWLEDGMENT. The investigations discussed in this paper were undertaken at the suggestion of Professor H. H. Whetzel, under whose direction and kindly criticism they were conducted and completed; for all of which, the writer wishes to express his most sincere appreciation.

Jensen proved, by infection experiments, that each suscept—oats, wheat, and barley—had its respective and specific loose-smut pathogene. To the smut fungi on oats he finally gave the name *Ustilago avenae.* He did not, however, at this time distinguish between the two kinds of oat smut. This distinction was made by Kellerman and Swingle (1890). They recognized the covered-smut pathogene to be distinct from that of the loose smut, and gave to it the name *Ustilago avenae,* var. *levis,* which was later raised to specific rank by Magnus.

For a brief but rather comprehensive sketch of this early history, one may consult Fischer von Waldheim (1869–70), Kellerman and Swingle (1890), and McAlpine (1910).

With the exception of the work of Lang (1913), which is discussed later, nothing of very great significance was added to the general fund of information and ideas from 1890 to 1922. During that period the results and conclusions of previous investigators were generally accepted, probably due to the fact that attention was being drawn more toward those diseases for which a satisfactory control had not been discovered.

PRE-ZADIAN CONCEPTIONS

Since the work of Zade has thrown new light upon the subject, which, in the writer's opinion, may be considered a turning point in the knowledge of the life history of *Ustilago avenae*, it may be well to coin the phrase *pre-Zadian conceptions* and under this as a heading recapitulate the status of the ideas on the subject up to 1922. Elaboration of details is limited here, only those essentials being presented which will serve to bring to memory the generally accepted conception of the life histories of these smut fungi. It will be evident that there are several conflicting ideas and that much of the earlier experimental procedure in obtaining data has not been in keeping with the course of events as they occur in nature, and, above all, that many of the conclusions are based on mere assumption.

LIFE HISTORY OF USTILAGO AVENAE

Inoculation

The source of inoculum is generally admitted to be the smutted plant in the field at flowering time. The dark sooty masses of chlamydospores in the diseased heads are disseminated by the wind and come in contact with the flowers of the healthy plant. It was held by Jensen (1888: 400–401) that only those spores lodging within the glumes of the healthy blossoms, which are open for the purpose of pollination, can play a significant rôle in infection. This was later confirmed by the work of Clinton (1900: 308–309), and is borne out also by the fact that

glumeless oats seem to suffer more than do glumed varieties. This, however, has been explained by Reed (1920 : 38) and others as being due to a more marked susceptibility of the glumeless oats.

Incubation

The incubation stage is held to be largely one of inactivity, extending over the period from blossoming time until shortly after the oats have been sown in the spring, the seed of the suscept and the spore remaining dormant together throughout the winter in storage. The spores as well as the oat seed may remain viable for a long time if kept dry. According to McAlpine (1910 : 104), Von Liebenberg germinated the chlamydospores at the end of seven and one-half years.

The active stage of incubation begins shortly after the oats have been sown. Moisture from the soil is absorbed by the seed and the spores alike, and if temperature conditions are favorable they both germinate. Kühn (1858 : 48–49) experimented with wheat and the stinking-smut pathogene, and came to the conclusion that penetration took place in the vicinity of the root node. Wolff (1873 : 660–661) was of the opinion that penetration could take place only through the young leaf sheath in the case of *Urocystis occulta* on rye. Kühn (1874 : 5–6) extended his experiments and concluded that penetration of *Ustilago carbo* (the name then used for the pathogenes causing both the loose smut of barley and that of oats) could take place in the root node, in the first stem node, and also in the internode between the two. Brefeld (1895 : 24–26), from spraying germinating oats with sporidia grown in artificial media, concluded that penetration of the axis of the young seedling is effected only in very young stages, and that infection does not result when the seedlings are thus inoculated after the inner leaves have pushed one centimeter through the leaf sheath. Lang (1913 : 177–178), working with serial sections, claims the infection court to be the mesocotyl. This, he says, elongates to push the young seedling out of the glumes. The spores germinate for the most part into germ tubes, but sporidia also are sometimes formed. At the end of seven days the mesocotyl is about one-half centimeter long. While the cells are still normally active the germ tubes remain on its surface, but when they begin to die (the mesocotyl being a short-lived structure) the germ tubes penetrate the dying cells on the surface and reach the healthy tissue beneath.

Infection

According to Brefeld (1895 : 32–37), the penetrating hyphae enter the seedling axis and grow directly through the tissues and reach the growing apex of the suscept. According to Lang (1913 : 178–179), the germ tubes, upon reaching the living cells inside of the dying mesocotyl,

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turn upward and grow between the cells, obtaining their food by means of haustoria. They traverse the primary node through an opening between the vascular bundle tips and the insertion of the first leaf. Here they branch and are thus established in the growing apex of the suscept. After the tissues of the primary node have become mature, the pathogene is not able to pass through the node and reach the meristem above. The mycelium originating from the hyphae that have successfully passed through the primary node, keeps pace with the growing point as this is pushed upward. The fungus causes a mild stimulation of the suscept cells, which accounts for the slightly more vigorous growth of the infected plants often observed during the period of optimum vegetation.

According to Butler (1918: 179–180), the growing point may sometimes grow away from the pathogene, and the mycelium thus left behind becomes disorganized, breaks up into segments, and disappears in the older tissues, just as it does even in the culms in which the pathogene succeeds in maintaining itself in the growing point.

As the culms elongate, the pathogene accompanying the growing point at last reaches the developing flowers. Here the mycelium branches profusely, becomes swollen and knobbed, and absorbs the differentiating suscept tissues. The chlamydospores are formed inside the terminal branches of the mycelium, the walls of which at length gelatinize and disappear. Thus, in place of the normal flower with its glumes, there is produced a mass of black or dark brown spores ready for dissemination. The degree to which normal tissues are replaced varies, sometimes both the flowers and the glumes being completely replaced, sometimes the glumes remaining partly or entirely intact. It is commonly observed also that only a part of the panicle is smutted, in which case it is always the lower part that is affected.

LIFE HISTORY OF USTILAGO LEVIS

The life history of *Ustilago levis* is held to be very similar to that of *U. avenae*. Much of this similarity has been assumed. The difference in appearance of plants smutted by *U. levis* and by *U. avenae* was brought out by Kellerman and Swingle (1890) when they distinguished between the two pathogenes. With *U. levis*, the heads of the oat plant are not so completely destroyed. The glumes remain intact and it is often difficult to know whether the spores are present without breaking open the glumes. Clinton (1900: 297) says, "These enveloping parts furnish such protection against dissemination of the spores that frequently the smutted flowers may be found in badly smutted grain after it is threshed." He states also that experiments with covered smut show that it is the spores which in the field have succeeded in falling between the open glumes at flowering time, that are blamable for infection.

Lutman (1910: 1200–1206), who produced artificial inoculation by spreading cultures of sporidia on young seedlings, emphasizes the fact that haustoria are absent, the pathogene being entirely intercellular. His description of spore formation is similar to that given above for *U. avenae*.

EPIPHYTOLOGY

Environmental factors governing both loose and covered smut have been assumed to be more or less alike.

Temperature

Low temperatures of the soil are held to favor both pathogenes. Spores germinate readily at temperatures as low as 10° C. At such a temperature oats germinate very slowly. Thus the pathogenes are able to penetrate and reach the growing point of the suscept before it emerges from the soil. If temperatures of 15° or above obtain, then the suscept grows so rapidly that the pathogene has little chance to become established in the tissues of the growing point. This idea was advanced by Brefeld (1895: 25), who, in all of his inoculation experiments, after spraying the young seedlings with sporidia kept them at a temperature of 10° for a period of several days before planting. Clinton (1900: 305) showed by experiment that late planting of oats reduces infection. Lutman (1910: 1201), working with *U. levis*, obtained infected plants by smearing sporidia on the young leaf sheath and keeping the inoculated seedlings at a temperature of 12° for from five to seven days. Heald (1919: 31), however, is of the opinion that late seedings produce more infection. Butler (1918: 180) also takes an opposing view with respect to temperature. He says, "When the temperature at sowing time is low, oats are more likely to escape smut, since the spores require a considerably higher temperature for germinating than the oat grains themselves." Tubeuf (1897: 286–287) clearly has misinterpreted Brefeld when he says Brefeld "found that oat-smut germinated best at 10° C., and not so well above 15° C." Brefeld did not find that the smut spores germinated best at 10° and not so well above 15°, but that effective penetration took place better at 10°.

On the one hand, then, Brefeld, Clinton, and Lutman believe that low temperatures favor infection, while Heald and Butler seem to think that high temperatures are favorable.

Moisture

Moisture relations may be particularly influential at two different periods in the life history of the pathogene—at the time of spore dissemination, and at the time of spore germination. It is obvious that a suffi-

cient supply of moisture is necessary at the time of germination of the seed and the spore. Clinton (1900: 308) is of the opinion that varying amounts of moisture may have a direct effect upon the amount of infection. He experimented with oats sown at different depths, and found that those broadcast resulted in 2 per cent of smut, those planted 1 inch deep resulted in 6 per cent of smut, and those planted 4 inches deep resulted in 10 per cent of smut. This he explains as being due in part to differences in soil-moisture content at the different depths.

At the time of spore dissemination, moisture plays an important part, for it is evident that heavy rains at this time will carry a large number of the spores into the soil and thus decrease the chance of inoculation. On the other hand, rain may aid in lodging the spores between the glumes. Jensen (1888: 405) noted that oats dipped in spore-charged water gave 29 per cent of smut, while those dusted with dry spores resulted in no infection. From this it would seem logical to assume that rains might tend to wash spores from the exterior of the glumes in between the closed glumes and the caryopsis, especially if the rains were light and came after a day favorable for spore dissemination.

THE LEIPZIG INVESTIGATIONS

ZADE'S STUDIES, 1922

As has already been intimated, some of Zade's (1922) observations and conclusions are not in keeping with the conception of the life history of *Ustilago avenae* as it has just been presented.

First of all, Zade confirmed the work of previous investigators in so far as his experiments showed that spores applied to the exterior of the glumes did not produce any appreciable amount of smut. He thinks, however, that the spores which lodge on the kernels of those oats that are deglumed during threshing, play a part in the usual percentage of infection. Spores applied after the glumes were removed gave much better results, but even here a maximum infection of only 26.9 per cent was obtained. Zade concluded that, although spores which lodge between the glumes can cause infection, the degree of infection is not sufficient to explain the frequency of epiphytotic attacks of the disease.

When he applied spores to the blossoms of oats at pollination time and subsequently examined them, *Zade discovered that almost all of the spores which fell on the stigma began to germinate at once* (in from fifteen to twenty hours, in the mild damp weather at the time of his experiment). The promycelia formed from the spores tended to be unusually long, but were otherwise normal and gave rise to budding sporidia. These sporidia produced hyphae which came in contact with the inner epidermis of the glumes and formed mycelium in the parenchyma beneath this epidermis. Zade obtained no evidence of flower infection

such as is known in *U. tritici*, the embryo remaining free from invasion and only the glumes being attacked. In fact, he says that *the mycelium does not penetrate the ovary and in no case can be found in, on, or under the epidermis of it*. He believes that the mycelium in the glumes is the most important source of inoculum for seedling invasion, although overwintering spores, or perhaps sporidia, may play a small part.

ARLAND'S STUDIES, 1924

The investigations of Zade were turned over to Arland (1924) for further prosecution. In the main Arland's results confirm those of Zade. He also inoculated oat blossoms at pollination time and noted that the spores germinated in the flower. This took place, however, in four days instead of from fifteen to twenty hours as had been observed by Zade. Moreover, the promycelia did not give rise to sporidia, the temperature at the time being above 30° C. This is in keeping with the recent observations of Bartholomew and Jones (1923: 575) and of Jones (1923: 590), who found 30° C. to be the maximum for sporidial production. At lower temperatures Arland noticed sporidial production in a few cases. Zade had suggested that resting sporidia might play a rôle in seedling infection, but Arland refutes this possibility since he was unable to keep dried cultures of sporidia viable for a longer period than six weeks. The ungerminated spores, however, do play an important rôle, in Arland's opinion, since he was able to get fairly high percentages of infection in certain variety tests when he introduced spores between the glumes of mature oats prior to sowing. He detected the development of mycelium not only in the glumes but also in the remains of the stamens and the stigma. *Incidentally he mentions that he found it in a few cases in the epidermis of the ovary.* By a more extensive study of the mycelium he found that it often broke up into biscuit- or dumbbell-shaped pieces which he called *gemmae*. He concludes that the seat of overwintering mycelium *is seldom the epidermis of the pericarp,* but is mostly the glume parenchyma and, without exception, the remains of the stamens and the stigma.

With naked oats which he dusted with spores at blossom time, Arland found that the mycelium which was produced wound itself among the hairs of the caryopsis. *It was seldom noticed in the epidermal cells.* He made variety tests with seed prepared in three ways: first, spores were placed between the glume and the kernel prior to the sowing of the oats; secondly, deglumed kernels were dusted with spores; and thirdly, sporidia were introduced between the glume and the kernel. The average percentage of infection resulting in the first test was 29 per cent, in the second 24.2 per cent, and in the third 5.1 per cent. *Avena nuda*, var. *chinensis,* the glumeless oat used, proved to be much more susceptible than the glumed varieties. *A. strigosa* and *A. brevis* were immune.

In tests on the comparison of smutting in plants produced from the inner grains of the spikelet with that in plants produced from the outer grains, Arland noted that the inner grains produced from three to four times, and in some cases from ten to twenty times, as many smutted plants as did the outer and larger grains.

ZADE'S STUDIES, 1924

Zade (1924) published a second paper which is a revised statement based on the investigations published by Arland. It appears that Arland, and also Diehl and Roesch, who published their theses later, were students working under Zade. Reference is made by the last two men to an unpublished thesis by Neumeyer, and, since Zade remarks that the blossom-inoculation work was carried out at his suggestion by Neumeyer, it is assumed that his paper (which the writer has not seen) covers in general the same investigations as are discussed in Zade's first paper (1922).

Since the foregoing account of Arland's studies covers all of the essentials given in the second paper by Zade, that paper is not discussed here in detail. It may be well, however, to mention the conclusions which Zade drew at that time. These were as follows:

The seat of the resting mycelium may be: (1) the parenchyma of the glumes; (2) *the epidermis of the pericarp, where the mycelium is very weak and only superficially or seldom present*; (3) in naked oats, the hairs of the caryopsis, where the mycelium is interwoven with them; (4) the remains of the anthers and the stigma, without exception; and (5) the remains of the lodicules, only seldom.

Zade believes that, in the case of naked oats, there is no possibility other than that infection of the seedling results from spores, and also mycelium and gemmae, which are on the epidermis of the caryopsis. This, he says, remains to be investigated. He states that he has suggested to Roesch (whose work is considered later) a method of inoculating glumes which will prove whether the resting mycelium and the gemmae are or are not actually the true inoculum for seedling invasion. So far, he says, this has only been assumed.

DIEHL'S STUDIES, 1925

Diehl (1925), continuing the work on oat smut and following in general the methods of Zade and Arland, came to the following conclusion:

Germination of the spores takes place most promptly on the moist stigma. If the weather is wet and cool, sporidia are produced; if it is warm and dry, the spores germinate directly into germ tubes. However, if the moisture within the flowers is sufficient, the spores may germinate even if they do not come in contact with the stigma. Only in

exceptional cases—that is, in very dry years—do the spores on the ovary remain ungerminated. In these cases, however, they can play a certain part in infection, even though a subordinate one.

In addition to finding mycelium in the glumes and the stigma and anther remains, Diehl found it in the lodicules and also observed that *it was very abundant in the epidermis of the ovary.* In the case of the glumes and the epidermis of the caryopsis, long hyphae were produced. Gemmae were formed in the stigma and anther remains, both from mycelium and from sporidia. In the latter case the sporidia merely enlarged and took on heavier walls.

Diehl noticed also a considerable difference in the manner in which blossoms opened for pollination. The blossoms of several varieties with which he worked did not open at all.

For the purpose of making tests of various disinfectants, Diehl removed oat glumes, sterilized them, and inoculated them with spores, thereby producing mycelium in them. He then returned these glumes to the kernels. In a like manner, he inoculated and brought about invasion of stamen remains and introduced these between the glumes. It is evident from this that he does not consider the mycelium in the pericarp as being very important.

ROESCH'S STUDIES, 1926

Roesch (1926), the latest of the Leipzig investigators cooperating on the studies on oat smut, pursued methods similar to those of his coworkers. His results and conclusions are as follows:

On the stigma, 100-per-cent germination of spores may take place. *On the epidermis of the caryopsis, the spores germinate very slowly and give rise to only a few short hyphae.* Ungerminated spores may be found occasionally on the epidermis and the epidermal hairs of the caryopsis. These do not play any significant rôle in infection, the resting mycelium and the gemmae on and in the remains of the stigma and the stamens, in the glumes, in the caryopsis epidermis, and in the lodicules, being concerned in the invasion of the seedling.

With naked oats the invasion of the seedling takes place only by the resting mycelium on the caryopsis epidermis, by gemmae, and, in isolated cases, by overwintered spores. Additional infection may also result from spores which reach the oats at threshing time and which germinate the next spring. Roesch considers that the pieces of stigma and anther remains with mycelium and gemmae, which he found between the epidermal hairs of naked oats, can play no important rôle in infection, since the hairs are broken off to a great extent during threshing.

Roesch made studies of seedling invasion in the following manner: Spores were germinated in a decoction of oat glumes. When there was

an accumulation of mycelium on the surface of the cultures, young seedlings produced from deglumed seed were dipped into the culture solution and thereby the sprouts and kernels were inoculated with mycelium. Roesch describes the invasion of the seedling in some detail. The hyphae penetrate the epidermis and the outer layers of cells by boring directly through them. In the inner tissues, however, the mycelium becomes intercellular. The protoplasm of the invading hyphae migrates with the hyphal tips and leaves empty walls behind in its path. Penetration was observed in the axis as well as in the coleoptile. In the latter, however, the penetrating hyphae, in most cases, did not enter as far as the third leaf. To determine in what stage of development the seedling is most susceptible, Roesch inoculated seedlings of different lengths, varying from 3 to 4 millimeters to 3 centimeters. After a period of three days, many relatively long hyphae were found in the younger seedlings while in the older ones hyphae were very scarce. The fungus seems to have the power to dissolve only the cellulose of thin cell walls. The susceptible stage of the young seedling expires when the first leaf breaks out of the coleoptile.

The pathogene can penetrate such fully immune varieties of oats as *Avena brevis*. In these cases the hyphae grow very slowly and produce plasma-poor threads, possibly as a result of unfavorable growth conditions. The pathogene may also penetrate even the seedlings of wheat, barley, rye, and oat grass. Further development of the hyphae, however, does not take place in these plants. With peas and beets, no penetration was observed.

In testing the same varieties during two different years, Roesch obtained no great differences in percentages of infection for the respective varieties, and therefore he concludes that susceptibility or resistance is an hereditary variety character.

By sowing some of his oats later in the season he noted higher amounts of infection. He advises, therefore, early spring sowing as a means of control.

The smaller inner grains produced higher amounts of smut, just as had been noted by Arland. Roesch believes this is due to the lower vitality of the inner grains, for he noticed that the plants developing therefrom grew more slowly in their early stages.

In several varieties of oats, Roesch also, as had Diehl, noticed considerable variations in the degree to which the blossoms opened for pollination. He concludes, therefore, that the possibility of inoculation is thus affected. Even in the same spikelet the second and the third flower open wider, and for a longer time, than the first flower. On this account Roesch recommends that the small grains be eliminated from the seed.

For the purpose of testing varieties, Roesch placed pieces of glumes

in petri dishes and inoculated them with spores. When mycelium had developed he inserted these pieces of glumes between the glumes of his seed oats. This was the method previously mentioned as being suggested by Zade. The highest percentage of infection resulting from the oats which were inoculated in this way was 26.4 per cent. In most of the varieties tested the average percentage of resulting smutted plants was less than 6 per cent.

COMMENTS ON THE LEIPZIG INVESTIGATIONS

The most outstanding contribution to our knowledge of the life history of *Ustilago avenae* since the pioneer investigations of Brefeld, Jensen, and Kellerman and Swingle, at least in the writer's opinion, has been this discovery of spore germination at blossoming time by Zade and his students. Brefeld's successful but artificial method of spraying young seedlings with sporidia grown in artificial culture was accepted as final confirmation of the conclusions of Kühn and Wolff in regard to the primary nature of seedling infection. The recent discovery of immediate spore germination has opened again what has been considered a closed chapter, and has especially stimulated the writer to seek further enlightenment on the life histories of the oat-smut pathogenes.

It may be recalled that Zade, Neumeyer, Arland, Diehl, and Roesch worked only with *U. avenae*. The life history of *U. levis* also needs investigation. Furthermore, it may be concluded that these German workers consider the mycelium and the gemmae in the glumes, and possibly in the stigma and anther remains, to be the chief inoculum for seedling invasion. Certainly they do not consider the mycelium in the epidermis of the caryopsis pericarp as being especially significant. Zade stated in his first paper (1922) that he would undertake to sow oats from artificially inoculated blossoms, of which part would be glumed and part deglumed. If his assumptions were correct, infected oat plants should come from the glumed oats, while the deglumed oats should give rise to healthy plants. So far this has not been reported as carried out. In his second paper, it will be remembered, he says that he suggested a method to Roesch whereby the glumes of oats might be inoculated and become infected. This method has been described in the discussion of Roesch's studies. Zade predicted that the stands of plants coming from such inoculated seed would show whether the resting mycelium and the gemmae in the glumes were really the true inoculum for seedling invasion, a theory which thus far had only been assumed. It will also be remembered that Roesch's results show a maximum percentage of 26.4 per cent of smutted plants, which was an exception to the general average of less than 6 per cent. In Zade's first paper he states that the 26.9 per cent of smut obtained by dusting spores on deglumed kernels is

insufficient to explain the epiphytotic attacks often occurring. If this is true, the 26.4 per cent obtained by Roesch offers no better explanation. This, it seems to the writer, throws some doubt on the possibility that the mycelium and the gemmae in the glumes play an important rôle.

THE CORNELL INVESTIGATIONS

BLOSSOM INFECTION AND SEEDLING INVASION

Before proceeding with a detailed discussion of the investigations carried on at Cornell University, it may be well to indicate the principal problem about which these researches center. This may be presented in brief by contrasting the conclusions of Zade and his colleagues with that of the writer on the point at issue.

On microscopic examination of blossoms which were artificially inoculated with spores of *Ustilago avenae* at pollination time, it was observed that the majority of the spores had germinated. No sporidia were produced, probably due to unfavorable temperature as suggested by the investigations of Arland (1924: 80), Bartholomew and Jones (1923: 575), and Jones (1923: 590). By frequent examination for several weeks after germination of the spores, it was found that mycelium is developed which penetrates and becomes established in the glumes (Plate II, 3), just as the German workers found. *On the other hand, mycelium was also found abundantly on, in, and under the epidermis of the caryopsis* (Plate I, 2). The presence of abundant mycelium in the pericarp is reported also by Diehl, as previously pointed out. Zade was unable to find such mycelium in any instance, and Arland noticed it only in a few cases. Roesch does not make particular mention of it. All of these investigators assume that the mycelium and the gemmae in the glumes and in the anther and stigma remains are the chief sources of inoculum for invasion of the developing seedling. *In the opinion of the writer, however, it is the mycelium developed on, in, and under the epidermis of the caryopsis that is accountable for most of the seedling invasion.* The data on which his conclusions as to the character of blossom infection and subsequent seedling invasion are based, are presented in the following pages.

Positional relation of pathogene structures to the oat flower and grain

Investigations by the writer were begun in June, 1924. It was recognized at once that accurate interpretation of the data would depend to a very great extent upon the ability to work with material (oats for seeding) the exact history of which was definitely known. Since no such seed was available, several preliminary studies were made with a supply of oats which had been harvested the previous year from a field known to have had considerable smut in it.

The most logical way to proceed seemed to be to determine first the fate of the chlamydospores introduced into the blossoms at flowering time, or, rather, the position in or on the seed of the structures of the pathogene which live over from blossoming time to planting. This was done by a process of elimination.

Five similar lots of seed (the term *seed* is used to designate the caryopsis with its enveloping glumes) from the supply mentioned above, were used. The first was treated with formalin; the second was untreated; the third was deglumed (the glumes being removed carefully with forceps); the fourth was deglumed and then about one-fourth of the stigma end of the caryopsis was cut off; and in the fifth, one-fourth of the stigma end of the entire englumed seed was removed. All five lots were sown at the same time, June 10, at the rate of 13 grams to a 15-foot row and approximately 1½ inches deep. The results are given in table 1:

TABLE 1. PERCENTAGE OF SMUTTED PLANTS RESULTING FROM DIFFERENT TREATMENTS

Treatment	Number of plants	Number smutted	Per cent smutted	Per cent of smut presumably due to parts removed
Formalin	600	0	0	
Untreated	600	40	6.67	
Glumes removed	692	28	4.05	2.62
Glumes and stigma end removed	486	16	3.29	3.38
Glume tips and stigma end removed	608	34	5.59	1.08

Since 2.62 is 39.28 per cent of 6.67 and may be assumed to represent the percentage of total infection due to parts of the pathogene in or on the glumes, then the remaining 60.72 per cent must necessarily come from pathogene structures in or on the caryopsis. Regardless of the nature of these parts— that is, whether they are spores or mycelium—*the fact that they were accountable for 60.72 per cent of all infected plants would indicate at once that mycelium in the glumes is not the chief cause of seedling invasion.* By subtracting the percentage due to pathogene structures in or on the glumes from the percentage due to those in or on the glumes and the stigma end combined, the amount due to pathogene structures in or on the stigma end alone may be obtained, namely, 11.4 per cent. This indicates that those pathogene structures in or on the

caryopsis which would account for 60.72 per cent of the infected plants, are not necessarily restricted to the stigma end.

These preliminary studies gave data which indicate only the position of the pathogene organs which are accountable for seedling invasion. In order to determine (1) the nature of these structures—that is, whether they are mycelium or spores—and (2) whether they are external to the caryopsis or within its tissues, more exacting experiments were undertaken.

Nature and relative importance of the various pathogene structures in the oat flower and grain

Scources of, and methods of selecting, experimental materials

Ustilago avenae.—As has already been stated, the writer considered it absolutely necessary to have seed of known history to work with. While the foregoing preliminary studies were being carried out, such seed was obtained in the following manner:

Supplies of Swedish Select, a glumed variety of oats, and Selection 202, a glumeless variety, were obtained from the Department of Plant Breeding at Cornell University. The Swedish Select oats were known to have come from an infested field. The lot of the glumed variety was divided into two parts, one of which was untreated and sown in plots at East Ithaca in order to guarantee a supply of spores for inoculation purposes. The other part, and the supply of the glumeless variety, were treated with formalin and sown in plots near Forest Home, which is about a mile from East Ithaca. These were to be used for producing plants for inoculation.

Shortly before the panicles emerged from the leaf sheaths of the plant at Forest Home, some of both varieties were covered with wax-paper bags to insure a supply of each kind against natural or accidental inoculation. They were harvested later, bags and all. Thus, clean uninoculated seed was obtained.

At blossoming time spores of *U. avenae* were obtained from the untreated plots at East Ithaca. These were examined microscopically to make sure of their rough-spored character, since covered smut also was present in the plots. The so-called "loose" character of the lesions caused by *U. avenae* does not seem to be consistent, since gradations may be found that correspond more to the "covered" character of the *U. levis* lesion.

When the blossoms in the plots at Forest Home were fully developed and undergoing pollination, some of both varieties were artificially inoculated. This was accomplished much after the method employed by Zade, namely, by tapping a small camel's-hair brush, previously dipped in spores, against the finger, thus introducing spore material into the

blossoms without touching any of the floral parts. Inoculated blossoms were tagged by means of colored strings. Flowers in a spikelet, not mature at the time of dusting, were removed with a pair of scissors.

Three days after dusting, flowers of each variety were examined microscopically. As has previously been stated, the majority of the spores had germinated. Those on the stigma seemed to have germinated first (Plate I, 1)—probably due to the sugary excretion as Zade has suggested; but, in addition, the spores lodged on the ovary walls were also fully germinated at the end of five days. This took place in both the glumed and the glumeless varieties. In no case, however, was sporidial production observed. The average temperature recorded by the United States Weather Bureau Station at Ithaca during this period was slightly under 30° C.

At the end of twelve days mycelium could be detected in the glumes and in the pericarp epidermis in both varieties. For detection of the mycelium the writer used at first an aqueous solution of eosin, but later, on becoming acquainted with a stain known as "cotton blue,"[2] he found it an easy matter to demonstrate the mycelium in both the glume parenchyma and the pericarp epidermis. This stain was used in all subsequent mycelial studies.

When mature, the inoculated oats were harvested and stored in paper bags under ordinary room temperature and moisture conditions. After the uninoculated oats were threshed, part of the glumed variety was dusted with spores without *first removing* the glumes, and part was dusted with spores after the glumes had been removed. A similar lot of the glumeless oats was dusted also. These supplies of inoculated seed were to be used for testing the relative ability of dormant spores, as compared with mycelium, to carry the pathogene over the winter. They were kept under exactly the same conditions as were the blossom-inoculated oats.

In the following spring, prior to sowing, the blossom-inoculated glumed oats were divided into two lots, one of which was deglumed to remove all pathogene structures which might be on or in the glumes.

In the spring of 1925 the writer had on hand, then, the following variously treated lots of seed oats, with definitely known history in so far as inoculation was concerned:

1. Swedish Select (glumed) uninoculated.
2. Swedish Select inoculated at blossoming time, glumes not removed and therefore with mycelium developed in the floral parts (possibly some ungerminated spores present).

[2]Cotton blue (Blue cotton, G-4 b., Soluble blue) was used as follows: Solution A, consisting of 50 grams of phenol, 50 grams of lactic acid, 50 cubic centimeters of glycerin, 100 cubic centimeters of distilled water. Solution B, the same as A, with 1 gram of cotton blue. Material from water was placed in A for a few minutes, then transferred to B for a few minutes, and then returned to A for clearing.

3. Swedish Select inoculated at blossoming time, glumes removed and therefore mycelium in the pericarp only (possibly some ungerminated spores present).
4. Swedish Select dusted with spores at harvest time, and therefore with ungerminated spores on the outside of the glumes.
5. Swedish Select dusted with spores after removal of glumes, and therefore with spores on the pericarp only.
6. Selection 202 (glumeless) uninoculated.
7. Selection 202 inoculated at blossoming time, and therefore with mycelium in the pericarp (possibly some ungerminated spores present).
8. Selection 202 dusted with spores after harvest, and therefore with ungerminated spores on the pericarp.

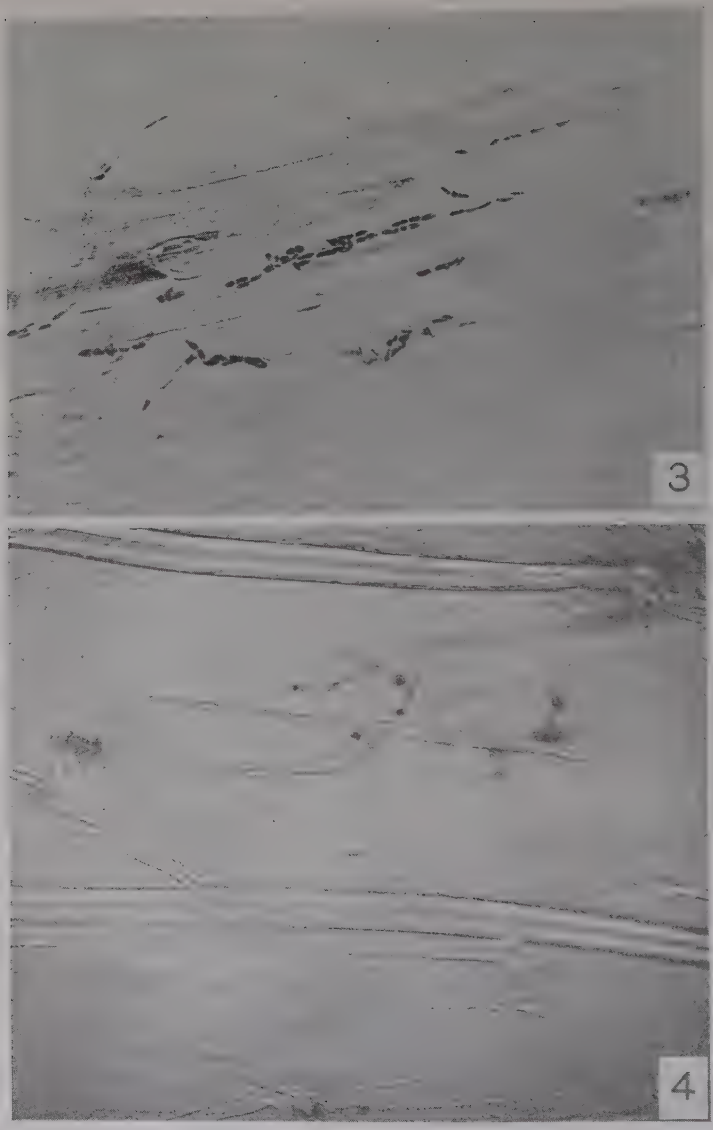
Ustilago levis.—In a like manner, lots of oats were obtained together with a supply of *U. levis* spores. Prior to inoculation the spores were examined as to their smooth-spored character. It may be well to note, however, that in obtaining spores for inoculating the first normal blossoms to appear, it was necessary to break open the smut sori. These covered sori do not open naturally until about the time when the last blossoms (largely from lateral shoots) are maturing. In the case of late-appearing smutted heads, the sori were not open at harvest time, and were broken open only by threshing. The possibility, then, of such spores entering the flowers lies in the fact that some of the first spores to mature are disseminated and lodge in the last of the flowers to open. Microscopic examinations following inoculation of flowers with *U. levis* spores disclosed germination and penetration of the floral organs identical with that described above for *U. avenae*. With glumeless oats the spores can, of course, come in contact with the caryopsis after pollination, or even during threshing. These points are discussed in more detail later.

The following seed supplies were obtained:

1. Swedish Select (glumed) uninoculated.
2. Swedish Select inoculated at blossoming time, glumes not removed and therefore with mycelium developed in the floral parts (possibly some ungerminated spores present).
3. Swedish Select inoculated at blossoming time, glumes removed and therefore mycelium in the pericarp only (possibly some ungerminated spores present).
4. Swedish Select dusted with spores at harvest time, and therefore with ungerminated spores on the outside of the glumes.
5. Swedish Select dusted with spores after removal of glumes, and therefore with spores on the pericarp only.
6. Selection 202 (glumeless) uninoculated.

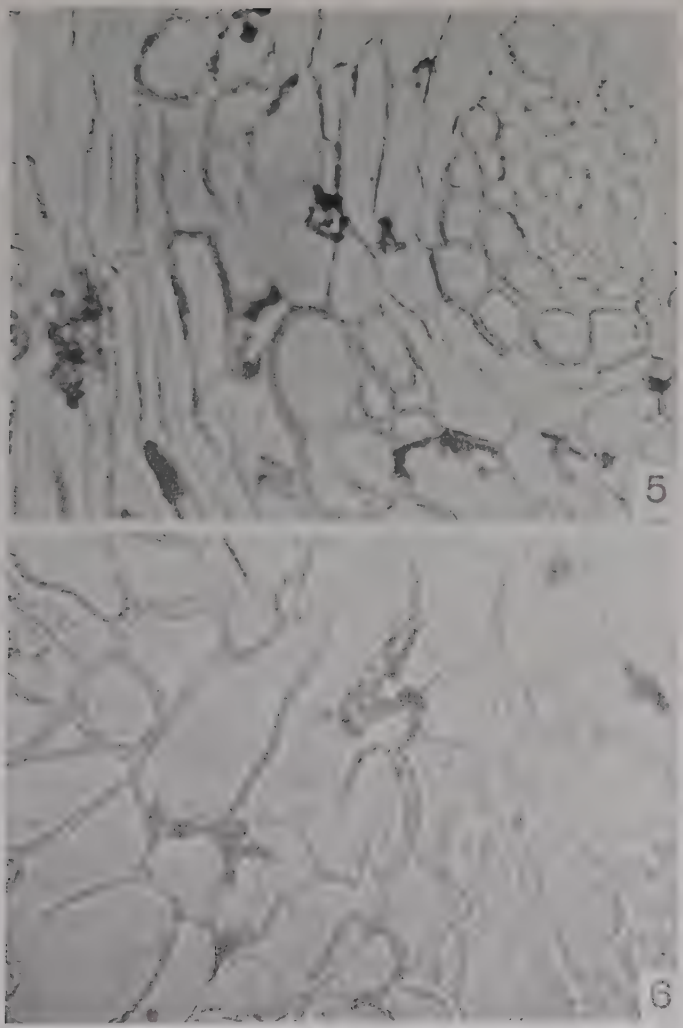
1, STIGMA BRANCHES OF THE OAT FLOWER (SWEDISH SELECT), SHOWING GERMINATION OF USTILAGO AVENAE SPORES AT POLLINATION TIME. ×225

2, EPIDERMIS OF THE PERICARP OF A MATURE OAT (SWEDISH SELECT), SHOWING THE MYCELIUM OF USTILAGO AVENAE IN, ON, AND UNDER THE CELLS. THE EPIDERMIS WAS STRIPPED FROM THE CARYOPSIS AND THE PHOTOGRAPH TAKEN FROM THE INNER SIDE. ×225



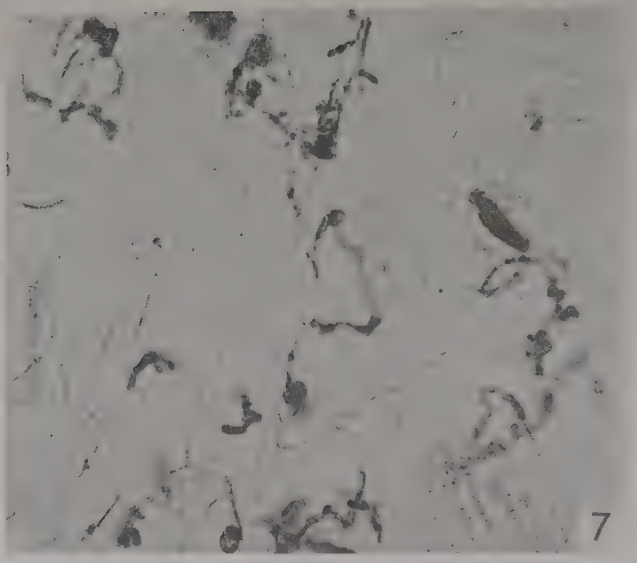
3, WING OF THE INNER GLUME OF A MATURE OAT (SWEDISH SELECT), SHOWING THE PRODUCTION OF "GEMMAE" FROM THE MYCELIUM OF USTILAGO AVENAE. ×265

4, EPIDERMIS OF THE PERICARP OF A MATURE OAT (SWEDISH SELECT), SHOWING GERMINATION OF SPORES AND PENETRATION OF THE CELLS WHICH HAS TAKEN PLACE IN STORAGE FROM SPORES APPLIED AFTER HARVEST. ×225



CROSS SECTIONS THROUGH THE NODAL REGION OF THE CULM OF A SMUTTED OAT PLANT (SWEDISH SELECT) SHOWING THE MYCELIUM OF USTILAGO AVENAE (5) IN THE PARENCHYMA CELLS BETWEEN THE VASCULAR BUNDLES, AND (6) IN THE SCLERENCHYMA CELLS ADJACENT TO THE VASCULAR BUNDLES. ×265

The photographs were made from sections which were cut rather thick. This was done to emphasize the abundance of mycelium present. The lighter parts of the mycelium in the photographs are those parts which were not directly in the plane of focus. Their appearance is not due to degeneration



CROSS SECTION THROUGH THE NODAL REGION OF THE CULM OF A SMUTTED PLANT (SWEDISH SELECT), SHOWING THE MYCELIUM OF USTILAGO AVENAE IN THE PITH PARENCHYMA. ×265

The photograph was made from a section which was cut rather thick. This was done to emphasize the abundance of mycelium present. The lighter parts of the mycelium in the photograph are those parts which were not directly in the plane of focus. Their appearance is not due to degeneration

7. Selection 202 inoculated at blossoming time, and therefore with mycelium in the pericarp (possibly some ungerminated spores present).
8. Selection 202 dusted with spores after harvest, and therefore with ungerminated spores on the pericarp.

Experiments and results

Early in June, 1926, the variously treated supplies of seed, both those inoculated with *U. avenae* spores and those inoculated with *U. levis* spores, were sown in the greenhouse under conditions favorable for the growth and development of oats. Both pots and flats were used. All seed was planted approximately 1½ inches deep, and all plantings received frequent watering. Under these conditions and the high temperatures of the greenhouse in July, the plants began to head out in early August. The results are given in tables 2 and 3.

TABLE 2. SEED INOCULATED WITH USTILAGO AVENAE

Lot	Treatment	Number of seeds planted	Number of plants developed	Number of plants smutted	Per cent smutted
1	Swedish Select uninoculated	225	222	0	0
2	Swedish Select inoculated at blossoming time; glumes not removed, and therefore with mycelium in the floral parts (possibly some ungerminated spores present)	225	212	142	67.0
3	Swedish Select inoculated at blossoming time; glumes removed, and therefore mycelium in the pericarp only (possibly some ungerminated spores present)	225	208	128	61.5
4	Swedish Select dusted with spores at harvest time, and therefore with dormant spores on the outside of the glumes	225	223	4	1.8
5	Swedish Select dusted with spores at harvest time after removal of glumes, and therefore with spores on the pericarp only	226	221	26	11.8
6	Selection 202 uninoculated	100	97	0	0
7	Selection 202 inoculated at blossoming time, and therefore with mycelium in the pericarp (possibly some ungerminated spores present)	80	79	46	58.2
8	Selection 202 dusted with spores after harvest, and therefore with dormant spores on the pericarp	100	97	12	12.4

TABLE 3. SEED INOCULATED WITH USTILAGO LEVIS

Lot	Treatment	Number of seeds planted	Number of plants developed	Number of plants smutted	Per cent smutted
1	Swedish Select uninoculated	225	222	0	0
2	Swedish Select inoculated at blossoming time; glumes not removed, and therefore with mycelium in the floral parts (possibly some ungerminated spores present)	80	77	52	67.5
3	Swedish Select inoculated at blossoming time; glumes removed, and therefore mycelium in the pericarp only (possibly some ungerminated spores present)	80	74	49	66.2
4	Swedish Select dusted with spores at harvest time, and therefore with dormant spores on the outside of the glumes	100	98	3	3.1
5	Swedish Select dusted with spores at harvest time after removal of glumes, and therefore with spores on the pericarp only	100	93	27	29.0
6	Selection 202 uninoculated	100	97	0	0
7	Selection 202 inoculated at blossoming time, and therefore with mycelium in the pericarp (possibly some ungerminated spores present)	80	77	47	61.0
8	Selection 202 dusted with spores after harvest, and therefore with dormant spores on the pericarp	100	96	23	24.0

Deductions

Since the nature and position of the pathogene structures in each case is known, it is an easy matter to approximate the amounts of seedling invasion due to the various pathogene structures in the seed.

Ustilago avenae (table 2).—It will be observed from lot 3, in which seedling invasion was due to mycelium in or on the pericarp and possibly some spores adhering to it, that 61.5 per cent of smutted plants resulted, which is 91.8 per cent of the total smut obtained in lot 2. Since examinations of samples of this seed showed that very few of the spores had remained ungerminated, very little, indeed, of this 91.8 per cent could have resulted from overwintered spores. Furthermore, when deglumed seed was dusted with spores (lot 5), in which case they received exceedingly larger quantities of inoculum than could possibly have entered the flowers in the field, a comparative percentage of only 11.8 per cent of smutted plants resulted. Even this 11.8 per cent was not due entirely to spores which remained ungerminated, for it was observed here, as well as in all cases in which spores were applied after harvest, that not only had many of them germinated in storage but penetration and invasion of the pericarp also had taken place. Moreover, the 1.8 per cent of smutted plants resulting from spores on the outside of the glumes (lot 4) confirms the already well-established conclusion that such spores also play an insignificant rôle.

With lot 7 of the glumeless variety, in which the smutted plants arose from mycelium in or on the pericarp and possibly some spores adhering to it, a percentage of 58.2 occurred. Here again, as was learned from previous examination of seed, very few spores remained ungerminated, and hence very little of the 58.2 per cent could have come from dormant spores. Lot 8, which received greater numbers of spores than would have reached the kernels in the field, contained 12.4 per cent of smutted plants.

In both the glumed and the glumeless oats it is very evident, then, that the majority of the smutted plants were certainly due to the mycelium in or on the pericarp of the seed. Overwintered spores, as well as any pathogene structures in or on the glumes, play a very subordinate part in seedling invasion.

Ustilago levis (table 3).—In lot 3, 66.2 per cent of smutted plants resulted from mycelium in or on the pericarp and possibly some overwintered spores adhering to it. This 66.2 per cent represents 98 per cent of the total smut, or the 67.5 per cent, obtained from lot 2. Since very few of the spores were observed to have remained ungerminated after the inoculation of the flowers, most of the 98 per cent of infection was due to mycelium and not to overwintered spores. This is emphasized also by the fact that when deglumed seed was dusted with spores at harvest time

(lot 5), in which case the seed received larger amounts of inoculum than did the blossom-inoculated seed, an infection of only 29 per cent resulted. The spores on the outside of the glumes (lot 4) produced only 3.1 per cent of smutted plants.

In lot 7 of the glumeless variety, 61 per cent of smutted plants were obtained from seed in which the mycelium in or on the pericarp, and possibly some adhering spores, were blamable. Since examination of this seed also showed that few spores do remain ungerminated after inoculation, the 61 per cent is certainly largely due to mycelium and not to spores. By way of contrast, spores dusted on the seed after harvest (lot 8) in amounts exceeding those applied at flowering time, resulted in only 24 per cent of smutted plants.

The procedure for *U. levis*, therefore, is practically the same as for *U. avenae*. The majority of the smutted plants are due to the invasion of the plants by mycelium already present in the pericarp of the seed from which they develop, and not from overwintered spores or pathogene structures in or on the glumes. It should be noted that in every case the resulting smut percentage for *U. levis* was somewhat larger than that for *U. avenae*.

Granting that some seedling invasion may result from spores which remain dormant, and also from mycelium in the glumes, it is still very evident that the majority of such invasions in both oat smuts are the result of mycelium developed in or on the pericarp from spores which lodge inside of the blossoms at pollination time. Careful examination of such seed revealed mycelium only in, on, or under the epidermis of the pericarp, and never in the embryo tissues. This absence of mycelium in the embryo is confirmed by the success obtained in controlling the disease by formaldehyde. If mycelium existed in the internal parts of the seed, the penetration of formaldehyde necessary to eliminate the pathogene would probably kill the seed also.

Since the formaldehyde treatment has been successful in the control of the loose smut of barley (Tisdale and Tapke, 1924: 264), it may be that the mycelium in the barley seed is also located largely in the pericarp. This has been suggested by these writers.

LIFE-HISTORY STUDIES

The evidence thus far has brought out the position, the nature, and the relative importance of the different pathogene structures which are present in or on the grain. Inoculation and incubation have been touched upon, but only in so far as was necessary for elucidation. For the sake of clarity, the writer believes that each phase of the life history of the two pathogenes—inoculation, incubation, and infection—should now be presented separately.

Inoculation studies

With Ustilago avenae

While natural inoculation was taking place, the writer spent considerable time not only in his own experimental plots but also in oat fields on the college farms, and made very careful observations. It was noticed that the spores of *U. avenae* are disseminated at a period beginning shortly before the healthy blossoms are open for pollination and continuing up until harvest. Most of the spores, especially those produced on the first, or main, culm of the smutted plant, are disseminated during the time when the first healthy blossoms are undergoing pollination, but the process is continued by later-appearing lateral shoots, and at harvest time there is still a considerable quantity of smut spores in the air.

The spores come in contact with all parts of the healthy plants, and a large majority, without doubt, either drop to the ground or are carried by the wind out of the oat field altogether. For the glumed-oat flower, there is a comparatively short period during which the spores may enter the blossom and come in contact with the floral organs. This period, the pollination period, varies approximately from twenty to sixty minutes, and as a general rule occurs during the late afternoon hours. Not all of the blossoms in a healthy panicle open at the same time, several days elapsing before all have undergone pollination. Spores, however, may lodge on the outside of the glumes at any time until harvest.

For glumeless varieties the inoculation period is very much longer. The spores may enter the blossoms at pollination time and continue to enter until harvest time. In addition, they may come in contact with the naked kernels at threshing time, when those spores which have not been disseminated in the field are shaken from the diseased heads that are harvested with the healthy oats.

With Ustilago levis

The inoculating process with *U. levis* is considerably different from that with *U. avenae*. The difference lies in the fact that with *U. levis* there is a much less developed correlation between the time of spore dissemination and the length of the pollination period. That this correlation may be more perfect under other climatic conditions than those that prevail in the area where these studies were carried on, is recognized. The sori containing the spores remain more or less intact until the majority of the blossoms have already undergone pollination. Even when the last blossoms are maturing, a very small number of spores are being disseminated.

With glumed oats, it will be readily seen that relatively few spores can reach the ovaries of the healthy flowers while they are still in the

field. Some few additional spores, however, probably do get inside of the glumes when the oats are being threshed. By placing oats in a bag along with *U. levis* spores, very little shaking is necessary to bring about such a condition, especially in oats the kernels of which are not tightly enveloped by the glumes, or in those with the glume tips broken off. With glumeless varieties, the spores may reach the naked kernels in large quantities at threshing time. Since very few of the spores of *U. levis* are disseminated in the field, this inoculation at threshing time would exceed that from the same process with *U. avenae*, in which very few of the spores are not disseminated in the field.

It is evident, therefore, that these observations confirm those of previous investigators, but perhaps greater emphasis may now be laid upon the differences existing between the inoculation processes in the two species.

Incubation studies

With Ustilago avenae

As has been stated, those spores of *U. avenae* that lodge on the stigma and the ovary in the glumed oats germinate in a very short time. Those on the stigma, due to the excretions thereon, give rise to germ tubes sooner than do those on the ovary walls, but under the weather conditions prevailing at the time of the writer's observations the majority of the spores were fully germinated at the end of five days. No sporidia were formed, a result which may be explained by unfavorable temperature relations as brought out by Arland.

Penetration of the glumes and the epidermis of the pericarp was evident. By removing the pericarp epidermis and staining with cotton blue, the developing germ tubes could be found in all stages. Some were found outside, some were entering the cells, and some which had penetrated through the cells were established between the epidermis and the adjoining tissues. As is well known, in the development of the caryopsis certain tissue changes take place in the inner part of the pericarp when it is fusing with the integuments of the ovule. The writer is of the opinion that the mycelium passing through the epidermis of the pericarp is favored by these changes. Careful examination of the material (Plate I, 2) brings out this point. The mycelium, once it has passed through the epidermal cells, seems to grow and spread very rapidly, and it appears as if no interference takes place by intervening cell walls.

With glumeless oats, the incubation process for those spores which reach the ovaries at blossoming time is exactly the same as with glumed oats. In addition, as is brought out later in the discussion of epiphytology, those spores which reach the caryopsis in the field after pollination also germinate in a very short time and produce mycelium which in-

vades the epidermis of the pericarp. This is true also of spores which do not reach the caryopsis until threshing time. The spores are capable of immediate germination, and favorable temperature and moisture relations for their germination usually prevail not only in the field but also in storage.

With Ustilago levis

It has been pointed out that relatively few spores of *U. levis* reach the ovary of glumed oats at blossoming time. However, those that do succeed in entering the latest blossoms to mature, behave as has been described for *U. avenae.*

In the glumeless varieties, the spores germinate, in all cases, shortly after they have reached the ovary or the maturing caryopsis. Without much doubt, this happens whenever they reach their goal while the oats are still in the field. The only exception to immediate germination would be cases in which exceedingly dry weather prevailed during the time of threshing, and when the oats were stored under exceedingly dry conditions. Examination of oats that had been dusted with spores shortly after threshing and stored in paper bags under ordinary moisture and temperature conditions prevailing in the office of the writer, showed a considerable amount of germination in the following spring (Plate II, 4). These points, however, are discussed more in detail later.

Infection studies

Since infection was found to be more or less alike in both loose and covered smut, the two processes are discussed together.

The time at which infection begins depends upon the time of penetration of the epidermal cells of the pericarp. This varies, as has been noted. However, the process is the same whether it begins shortly after pollination or as late as storage. As has been described under incubation, the mycelium passes into and under the epidermal cells. Just how much of this is to be included in infection it would be difficult to say, as no definite point marking the end of incubation and the beginning of infection can be established because no visible effect on the suscept tissues has been observed.

If infected seed is examined in the spring, it is seen that the mycelium appears to have developed to a considerable extent during the winter and can be found abundantly under or in the epidermis of all parts of the pericarp. It is evident, then, that the mycelium does not stay dormant throughout all of the winter, but under favorable conditions may be slowly growing and extending itself. The difference in the amount of mycelium at harvest time and in the spring confirms this conclusion.

In the spring, the mycelium thus developed in the pericarp brings about invasion of the young developing seedling. Just how this takes

place will necessitate further study than has been made by the writer. The observations of Roesch (1926:387) must be discounted, since his methods are not in keeping with natural conditions. From the limited investigations that have been made by the writer, it would seem that the mycelium grows directly into the mesocotyl of the seedling. Since it is difficult to section material and retain the epidermis of the pericarp in place, a direct connection between a strand of mycelium in it and a strand in the seedling has not been observed. On the other hand, the very youngest seedlings in which mycelium could be detected were about seven days old and the invading mycelium was found only in the mesocotyl—that is, between the node from which the coleoptile develops and the node from which the first roots arise. In seedlings a little older, from twelve to fifteen days, mycelium was found in the coleoptile node and also extending a short distance down into the roots.

Should it be found that the mycelium grows out of the pericarp—that is, that it develops on the outside of the caryopsis prior to its entrance into the seedling—then the pathogene would have to be considered as having a two-cycled life history.

On examination of the culms of mature plants bearing smutted heads, mycelium is seen to be most abundant in the nodal regions and in the base of the leaf sheaths. It does not seem to favor any particular tissue, being present in the parenchyma of the pith (Plate IV, 7) as well as in that surrounding the vascular strands (Plate III, 5) and in that of the leaf sheaths. In addition it is frequently present in the sclerenchymatous tissues (Plate III, 6). It is prevailingly intracellular, although often found between the cells. This is contrary to the statements of Butler (1918:180) and Roesch (1926:389), but confirms those of McAlpine (1910:9). No true haustoria could be detected. In the internodes the mycelium is rather sparse and the hyphae are long and unbranched. In the nodes it is abundant and the hyphae are short, convoluted, and much branced. There is no evidence of mycelial degeneration and disappearance in the older or lower parts of the culms, as stated by Butler (1918:180). In fact the mycelium seems to be most abundant in the lowermost nodal regions.

The mycelial invasion of the developing inflorescence and the formation of chlamydospores has not been studied by the writer.

EPIPHYTOLOGICAL STUDIES

The effect of temperature and moisture conditions was recognized very early as having considerable influence upon the infection of oats by these smut pathogenes. The difference in opinion as to whether low temperatures or high temperatures favor seedling invasion is explained by the recent work of Bartholomew and Jones (1923), from whose in-

vestigations it appears that both extremes of temperature are unfavorable to the disease, the high, however, more than the low, and that maximum percentages of smutted plants result at from 18° to 22° C. Furthermore, these investigators show that low soil moistures, within a certain range of temperatures, favor invasion, while high soil moistures accompanied by high temperatures result in complete elimination of the fungus. The work of Reed and Faris (1924) is also important, since it emphasizes the fact that soil moisture, soil temperature, and soil reaction, interact as a set of factors, any one of which may be limiting. Statements with regard to early or late planting mean nothing unless they are accompanied by temperature and moisture records.

The experiments of the above-mentioned investigators, however, were carried on with oats which were dusted with spores just prior to sowing. Since seedling inoculation by spores can now be considered as taking place only in exceptional cases in nature, new data should be sought to explain the effects of environmental factors on seedling invasion. The writer has conducted a few experiments, which, although not conclusive, add something to the general knowledge.

First of all, dehulled and hull-less seed which had been dusted with spores just after harvest and which were subsequently examined in the spring, showed that the majority of the spores had germinated (Plate II, 4). Furthermore, penetration of the pericarp by germ tubes was evident also. From this it may be concluded that there are temperature and moisture conditions under which the pathogene may develop, but which are not conducive to the germination of the oat. Confirmation for this was sought in the following way:

Spores of *Ustilago avenae* and *U. levis*, on slides, were placed along with oats in petri dishes lined with wet filter paper. Both glumed and glumeless varieties were used. One set of dishes was kept for ten days at a temperature of 6° C., another at 10°, and a third at 18°. At intervals during the ten days the oats and the spores were examined for germination. In the set kept at 6° the majority of the spores had germinated, but only a very few of the oat seed had sprouted, and in these few cases the sprouting consisted of mere emergence of the radicle. In the set kept at 10° the spores had likewise germinated, and germination of the oats seemed to be taking place but at an exceedingly slow rate. At 18° both the spores and the oats had germinated rapidly. It is evident, then, that smut spores may germinate at temperatures well below those favorable for oats. (The term *germination* is used by the writer to mean emergence of the germ tube or the oat sprout with continued growth.) This does not support Butler's view, namely, that the spores require a considerably higher temperature for germination than do the oats, if his interpretation of oat germination is the same as that of the writer.

Similar experiments were conducted in which moisture was made the limiting factor. Six petri dishes were prepared with dry filter-paper linings. Spores and oats were introduced as in the previous experiments. To the first dish no water was added; to the second, one drop; to the third, three drops; to the fourth, six drops; to the fifth, nine drops; and to the sixth, twelve drops. The dishes were kept at a temperature of 18° C. for ten days. At the end of this time they were examined, and it was found that the spores had germinated in all dishes in which three or more drops of water had been supplied. In the case of the oats, only a few of those supplied with as many as twelve drops of water showed any germination at all. It is evident from this that spores are capable of germinating under moisture conditions which are deficient for the sprouting of the oat.

These experiments were followed by similar ones in which the spores were dusted directly upon the deglumed and the glumeless oat kernels. With temperature as a limiting factor, it was found that at 6° C. not only spore germination but also penetration of the pericarp epidermis took place, while the oats failed to develop sprouts. With moisture as a limiting factor, spore germination and penetration took place when three or more drops of water were added to the petri dishes, but below twelve drops the oats did not show any sign of germination. The amount of pericarp penetration was much greater than with temperature as a limiting factor.

From these data, it seems to the writer that one may be justified in concluding that infection may take place in storage by spores which reach the oats at threshing time. The ordinary manner in which oats are usually stored would certainly supply the necessary conditions of moisture and temperature.

In those experiments whereby the writer determined the position and the nature of the pathogene structures which carry the pathogene over winter in the seed, some of the blossom-inoculated seed did not produce plants with smutted heads. Examination of the culms of some of these revealed the fact that mycelium was present in their lower parts. Seedling invasion, then, had taken place, but for certain reasons the pathogene had not reached the inflorescence of the plant. This mycelium was found in most cases to be confined to the first node, but in a few cases it had reached the second and the third node. Since these oats were grown under conditions most favorable for rapid germination and development, it would seem that invasion of seedlings should universally result from infected seed. The importance of this lies in the fact that smutted *panicles can no longer be considered as a criterion of infection,* especially if variety tests are made in the field where absolute control of the plants is impossible. There might easily exist non-inheritable differ-

ences in some individual seeds which would effect faster germination. It will be remembered that Arland and Roesch noted that the inner and smaller kernels of a spikelet produced more smutted plants than did the outer and larger ones. That resistance of certain oat varieties may be explained by physical rather than chemical differences is also suggested.

As a further bit of evidence to show that smutting of panicles depends upon growth relations of suscept and pathogene, the following data are presented: Oats were sown and were permitted to attain a height of approximately six inches. They were then carefully transplanted after smut spores had been applied in the region of the first node. By infrequent watering a very slow growth was maintained. The plants resulting were rather stunted, but smutted panicles were produced in six of the nine thus inoculated. Checks were normal. Although such conditions would probably never take place in nature—except when the pathogene had failed to reach the growing point of the suscept at germination time, but succeeded in catching up due to an ensuing period of slow growth on the part of the suscept—this experiment provides additional evidence that smutting of panicles is dependent upon the growth relations of the suscept and the pathogene. It also refutes the statement of Lang (1913: 179) and of Butler (1918: 180), that the mycelium is halted by the maturing of nodal tissues.

Inasmuch as successful inoculation has been obtained by dusting spores on deglumed and glumeless seed prior to sowing, it may be well for the writer to present an explanation of why this is possible when under natural conditions seedling invasion is due to mycelium existing in the pericarp of the oat from which the seedling develops.

As has been pointed out, most investigators who have worked on seedling inoculation with spores recognized the fact that success depends to a large extent upon keeping their spore-dusted seed or seedlings at a low temperature prior to planting. Under such conditions the germination of the oat is retarded, while the spores germinate and produce germ tubes (or sporidia which in turn produce germ tubes), and then invasion proceeds as it would have done if mycelium had already existed in the pericarp. The process, after germ tubes are produced, is practically the same as would have occurred under natural conditions, but, since it takes several days for this development, the process as a whole is very uncertain. If the spore-dusted seed is immediately sown in the field, then the chances of infection which would give smutted panicles are decidedly lessened and not at all in keeping with nature. This has probably been the procedure in the major part of the work that has been done on varietal susceptibility, and, as has been indicated, the use of smutted panicles as a criterion of infection must be considered as a grave source of error in such work.

Aside from growth relations of pathogene and suscept, emphasis should be placed on another factor which has perhaps been overlooked or at least underestimated. In the case of glumed oats and *U. avenae*, it has long been recognized that only those spores which enter between the glumes and the ovary play a significant rôle as inoculum. Very frequently, in damp, cool weather, oat blossoms fail to open for pollination. The writer considers this to be a factor of great importance in the explanation of seasonal differences in amounts of smut.

GENERAL DISCUSSION AND CONCLUSIONS

To a certain extent, conclusions have already been indicated from the various data presented, but there are certain outstanding generalizations which should be more or less emphasized.

In the light of the discovery of spore germination at pollination time by Zade and his students, and the investigations of the writer, it is evident that the current conception of the life histories of *Ustilago avenae* and *U. levis* will have to be altered in several important respects.

In the first place, it will be necessary to remove the oat-smut pathogenes from the commonly called "seedling infection" group and place them in the "flower infection" group. Since they differ from the pathogenes in the latter group, however, in that the embryo is not invaded, the mycelium being restricted to the pericarp, it would seem that some distinction should be made. If a continuation of such groupings is advisable, the writer suggests that the "flower infection" group could be divided into an "embryo infection" subgroup and a "pericarp infection" subgroup. The oat-smut pathogenes could then be placed in the latter subgroup.

In addition, it must be recognized that infection of the pericarp is not restricted to the blossoming period of the oat. With *U. avenae* and glumed varieties of oats, this is largely the case. With *U. avenae* and glumeless varieties, infection may take place in the field at any time after pollination and even during storage of the oat. Since most of the spores are disseminated in the field, most of this infection would probably take place prior to harvest. With *U. levis* and glumed oats, the pericarp infection must be confined largely to the blossoming period. With glumeless varieties, it doubtless takes place also after the grain has been threshed since most of the spores reach the oats at threshing time, and therefore it is highly probable that much of the infection ensues in storage. In general, the length of the period during which inoculation is possible is without doubt the most important factor concerned with the resultant amounts of smut. All these points are in keeping with the writer's observations on the relative amounts of loose and covered smut during the years 1924 and 1925. Loose smut seems to be far more prevalent than covered smut among the glumed varieties of oats, while

glumeless varieties have presented greater percentages of both kinds of smut but with the covered smut predominating.

Since the inoculation of glumed varieties of oats must, for the most part, take place during the period of pollination, it would seem that a failure of the blossoms to open for pollination would be a most important controlling factor in the amount of smut in the succeeding crop. This must very often take place, because either cool or damp weather at blossoming time prevents opening of the flowers.

In regard to other phases of environmental influence, further emphasis may be placed on the fact that temperature or moisture cannot be considered alone in an attempt to explain varying amounts of smutted plants in the crop. As is necessary in many other physiological studies, all factors which may influence the process must be taken into consideration before the effect of any one may be designated as limiting.

Of special importance is the fact that smutted panicles cannot be considered as an infallible criterion for infection, unless tests are made under conditions where absolute control of environmental factors is maintained throughout the entire development of the suscept. This, it would seem, is impossible under field conditions.

Considerable doubt necessarily arises as to the value of certain existing data on varietal susceptibility of oats to smuts. All work along this line should be checked by inoculating oats in accordance with the manner in which it happens in nature. If the artificial way of dusting seed with spores is continued, modifications should be introduced. The glumes should always be removed prior to dusting, and the inoculated seed exposed to conditions which will bring about that which would exist in nature, namely, infection of the pericarp before the oat starts to germinate. Comparisons of percentages of infection of naked and glumed varieties are worthless if spores are applied to the latter without first removing the glumes. Since there certainly must be differences in varieties which would influence the possibility of spores entering their blossoms, as observed by Diehl and Roesch, methods of inoculation approximating that in nature might change considerably the present status of our knowledge of varietal susceptibility.

SUMMARY OF CORNELL INVESTIGATIONS

1. Spores which reached the stigma of the oat flower at pollination time germinate at once or within a few days. This happens in glumed and glumeless varieties of oats, with both *Ustilago avenae* and *U. levis* spores.

2. Spores lodging on the ovary walls also germinate in a very short time.

3. With glumed oats the inoculation period is rather short, being restricted to the time during which the glumes are open for pollination.

Since some glumes are broken off by threshing, inoculation may take place to a certain degree at that time. With glumeless oats the inoculation period extends from pollination time until and during threshing.

4. The success of inoculation is dependent to a large extent upon the time of spore dissemination and the length of the period during which the spores may reach the ovaries or the caryopses. *U. avenae* spores are disseminated over a period extending from shortly before pollination until harvest. They must reach the ovaries of glumed varieties of oats during the time when the flowers are open for pollination, but may lodge upon the ovaries or the caryopses of glumeless varieties at any time after the flowers have opened. *U. levis* spores are not disseminated to any great extent until the very last of the flowers are being pollinated. With glumed varieties the possibility of the spores entering open flowers is very much less than for *U. avenae* spores. With glumeless varieties the majority of *U. levis* spores reach the caryopses of the oats during threshing.

5. Mycelium resulting from the spores germinating at blossom time may penetrate the glumes, but this mycelium is insignificant or at least unnecessary as a source of inoculum for seedling invasion, being entirely wanting in the case of glumeless oats.

6. Mycelium resulting from spore germination during the blossoming period or the maturing period of the oat, or even when the oats are in storage, penetrates and invades the pericarp of the caryopses and becomes established in and under the epidermal cells.

7. *The mycelium in the pericarp is accountable for most of the seedling invasion.*

8. The success of invasion resulting in spore formation (smutted spikelets) is dependent upon certain combinations of environmental factors, rather than on any specific one. In general, slow germination of the oat and continued slow development of the plant favors the pathogenes. Rapid germination and rapid growth of the plant, although they do not prevent seedling invasion and infection of the lower part of the plant, will often result in normal production of flowers.

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